PAPERS READ.

OBSERVATIONS ON THE POISONOUS CONSTITUENTS OF THE VENOM OF THE AUSTRALIAN BLACK SNAKE *(PSEUDECHIS PORPHYRIACUS)*.

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With the exception of a few observations, I can find no record of investigations into the chemistry of the venom of the Australian snakes such as have been undertaken with the poison obtained from Indian and American species.

The first investigation into the chemistry of snake poisons of any importance was by Prince Lucien Bonaparte with the venom of an adder *(Pelias berus)* in 1843. An interesting account of this is given by Sir Joseph Fayrer in a paper in the Proceedings of the Medical Society, London, 1884.

Bonaparte found that the activity of the poison was associated with the portion coagulable by alcohol, and gave the name of viperine to this coagulated material.

In the first volume of the Analyst (1876), Winter Blyth states that he found in cobra poison a crystalline highly poisonous body, to which he gives the name "cobric acid," and that this is the sole poisonous constituent. Blyth's conclusions are criticised by Wolfenden (Journal of Physiology, Vol. vii.) who at the same time shows that the toxic qualities of cobra venom are resident in its proteid constituents.

In 1878 Professor Pedler,* of Calcutta, published an account of his investigations. He made an ultimate analysis of the dried poison, and showed that in percentage composition it closely corresponded with that of albuminous bodies generally. He also claimed to have separated a "semi-crystalline" body of an "alkaloidal nature," to which he ascribed the potency of cobra venom.

* Proc. Roy. Soc. 1878.

Armstrong,* and still earlier Dumas, had made ultimate analyses with similar results.

In 1883 Wall published a very interesting book, "Indian Snake Poisons, their Nature and Effect," in which are two facts of special importance, viz.:—(i) That the whole of the poisonous properties reside in the coagulum by absolute alcohol, and that if the alcohol be absolute, the filtrate is innocuous. (ii) That the poisonous principle is taken up by distilled water from this precipitate by alcohol, and that the solution so obtained possesses all the properties of cobra poison.

Since then three papers by Norris Wolfenden† have appeared in the Journal of Physiology. In these papers Dr. Wolfenden establishes the proteid nature of the poison, and excludes the possibility of alkaloids, ptomaines, germs and any body of the nature of cobric acid. He claims to have separated an albumen, an albuminate, and a globulin from cobra venom, to all of which he ascribes poisonous properties.

Investigations into the nature of the poisons of the American rattle-snakes, the mocassin and copper-heads have been carried on by Drs. Weir Mitchell and Reichert, whose results appeared as a preliminary report in the Medical News, Philadelphia, 1883.

A complete account was published by the same authors in the Smithsonian Contribution to Knowledge for 1890.

They prove the proteid character of snake poison in American snakes, and ascribe poisonous properties to three varieties of globulin, which they separated by "appropriate processes," and a peptone. The reactions given by their so-called peptone are characteristic of that class of bodies which we now know as albumoses.

With the idea of determining the presence of albumoses, in the venom of our Australian snakes, I proceeded in the following manner.

I placed the poison from the ducts, and the squeezings from the glands, of two black snakes under a large volume of absolute alcohol for three months. By this treatment the whole of the

* Snake Comm. Rep. 1874.

† Journ. Physiol. Vol. vii.

proteid was precipitated, and all proteids except albumoses and peptones rendered insoluble.

The alcohol was filtered off and allowed to evaporate very slowly under a bell jar, at the ordinary temperature of the laboratory. This alcoholic extract I shall refer to as solution A.

The precipitate, after the adherent alcohol had been driven off by evaporation at 40° C., was treated with 1% NaCl solution for some hours and filtered. This solution of precipitated proteids I shall refer to as solution B.

By these procedures any body of the nature of an alkaloid, ptomaine, or Blyth's hypothetical cobric acid, would be in solution A, and those proteids not rendered insoluble by the prolonged sojourn under alcohol in solution B. All germs, if such were present, would be destroyed.

Examination of Solution A.

The solution is yellow in colour, with a disagreeable pungent smell, and of a marked acid reaction.

During the slow evaporation of the solution no crystals appeared. The lower portion of the fluid was repeatedly drawn off and examined under the microscope with negative result.

The residue after evaporating off the alcohol was dark brown in colour, greasy, and of an acid reaction. The acid present in it was freely soluble in alcohol and ether. Litmus paper reddened by either of these solutions returned to the neutral tint on drying.

This alcoholic extract contained no proteid and the whole of it was mixed with a little salt solution 7% and injected into the peritoneal cavity of a guinea pig without result.

Examination of Solution B.

This solution was clear, colourless and neutral, and although it was a very weak one, 1 cc. injected into the jugular vein of a guinea pig caused its death in 23 minutes with the usual symptoms of snake poison. The remainder was then submitted to the following chemical tests :—

(1.) Boiling. No coagulation.

(2.) Nitric acid. Slight turbidity, on the addition of salt a precipitate, which disappeared on warming and re-appeared on cooling.

(3.) Copper sulphate and potash. Rose buiret.

(4.) Saturation with magnesium sulphate. Causes a precipitate.

(5.) Saturation with ammonium sulphate. Causes a precipitate.

Of a small test tube $\frac{1}{4}$ inch was filled with the crystals of $MgSO_4$ or $Am_2\ SO_4$ and then the solution poured on to them so as to cover the crystals by $\frac{1}{8}$ inch. The tubes were then allowed to stand 24 hours in a warm place. At the end of this time the crystals in both tubes were found to be covered with a layer of flocculi.

(6.) Of the solution 2 cc. were shaken for 24 hours with $Am_2\ SO_2$ crystals, to which one drop of 5% $H_2\ SO_4$ had been added* and then filtered.

The filtrate was proteid free.

On account of the minute quantity at my disposal I have not been able to accomplish more than this at present, but these few experiments enable me to answer in the affirmative the question whether our snake poison contains poisonous albumoses.

The question whether any other proteid constituents of snake venom, not separated by these means, possess toxic powers (*e.g.*, albumins or globulins), is so far unanswered.

I am at the present time, in conjunction with Mr. T. McGarvie Smith, conducting a systematic investigation into the chemistry of snake poison. The great difficulty we have to contend with is the scarcity of material. For though we have reason to believe that the virulence of the poison of the black snake is as great as that of the cobra, the amount of poison voided at one time by the latter snake is 10 or 20 times as great as that procured from the largest snakes we have had in our possession, some of which have been remarkably fine specimens.

In conclusion, I take this opportunity of acknowledging my indebtedness to Mr. Smith, whose kindness enabled me to compare the effects of the injection of my isolated albumoses with those produced by the fresh poison.

* Vide Neumeister, Zeitscrift f. Biologie, Bd. xxvi.